REMARKS

By this amendment Claims 4, 5, 7, 8, 10, and 32 have been amended. Claims 1-21, 25-27 and 31-33 are currently pending.

Claims 4, 5, and 32 are amended in accordance with Examiner's suggested amendments.

Claims 7, 8, and 10 are voluntarily amended so as to be consistent with the language of claim 1 and to derive proper antecedent support from claim 1.

Claim rejections under 35 U.S.C. 112, second paragraph

In Item 4,The Examiner has rejected claims 4, 5, 32, and claim 6 by virtue of dependency from claim 5, under 35 U.S.C. 112, second paragraph, contending that these claims are indefinite.

Specifically, the Examiner states that:

- claim 4 and 5 are indefinite due to the recitation "of induction medium" at the end of these claims, and suggests that deletion of this phrase would obviate this rejection;
- claim 4 is indefinite in the recitation of "a said arabinogalactan protein", a suggests that deletion of "a" from this phrase would obviate this rejection; and
- in claim 32, step (d) it is unclear to what "15%" is referring, and suggests that insertion of the phrase "viable microspores after a 10 day incubation period" at the end of step (d) would obviate this rejection.

Applicants have amended claims 4, 5, and 32 in accordance with Examiner's suggestions so as to obviate rejections of these claims, as well as dependent claim 6. Therefore, removal of each of Examiner's rejections under 35 U.S.C. 112, second paragraph is respectfully requested.

Claim rejections under 35 U.S.C. 103(a)

In response to Examiner's presumption, **in Item 5** of the Action, relating to common ownership of claimed subject matter by joint inventors, applicants confirm, in compliance with 37 CFR 1.56, that the claims of the present invention were commonly owned by the inventors.

In Item 6, the Examiner has rejected claims 1-14, 18-21 and 31-33 under 35 U.S.C. 103(a), alleging that these claims are obvious having regard to the combination of US 5,445,961 (Genovesi) and EP 0 455 597 (Kreuger). Applicants respectfully disagree with

Examiner's allegation and traverse Examiner's rejection on the basis of, at least, the two following arguments.

Within Examiner's rejected claims, claims 1, 18, 31, 32, and 33 are independent, and therefore the following arguments will be provided in respect of these claims.

Applicants initially note that Examiner's assertion (page 4 of Action, second paragraph) that Genovesi teaches that "microspores are maintained at a uninucleate cell cycle G1 phase" is incorrect, as the G1 phase is not mentioned in Genovesi.

First Argument

Applicants contend Genovesi and Kreuger are not analogous art, or if considered analogous there is no motivation to combine these two references (see 'Second Argument' below). However, even if Genovesi and Kreuger are combined, applicants submit that the combination does not teach every element of the claimed methods of the present application, and it would not be obvious for a person skilled in the art to make up for the deficiencies of the combination.

The independent claims of the present application, each recite "from about 50% to about 100% of microspores at a uninucleate stage of development".

Kreuger only refers to microspores in passing, makes no mention as to number of microspores at a uninucleate stage, and provides no guidance as to protocols incorporating AGP for microspore culture.

Genovesi indicates that uninucleate microspores are preferred (see for example paragraph bridging Col 4 and Col 5). Furthermore, Genovesi discloses in Example 3, Table II (Col 22, lines 20-35) that from about 50% to about 100% of microspores at a uninucleate stage may be obtained. **However, in the same table, Table II (reproduced below), Genovesi clearly teaches that such a level of uninucleate microspores can only be achieved with the addition of colchicine.**

TABLE II

	Percent of Uninucleate and Binucleate Microspore Divisions Induced by Colchicine.		
	% Colchicine	% Uninucleate	% Binucleate
		Experiment A:	
	0	14	86
	0.01	21	79
→	0.05	74	36
		Experiment B:	
	0	16	84
	0.01	25	75
→	0.025	66	34
→	0.05	70	30
→	0.1	60	40

Arrows have been added in the left hand margin of Table II to identify the treatments that result in 50% to 100% uninucleate microspores.

Table II summarizes results from two experiments disclosed in Example 3. The preculture conditions of Example 3 are specified (at Col 22, lines 9-13) to be those of Example 2 with the only modification being addition of various amounts of colchicine. The preculture conditions of Example 2 comprise:

- 4 days in no media + 10 days in media;
- ascorbic acid;
- culture at 10°C; and
- mannitol.

In Table II, the results of Experiments A and B establish the requirement for colchicine in that:

- absence of colchicine only yields 14% (Exp A) and 16% (Exp B) of microspores that are uninucleate; and
- only those precultures having a colchicine concentration greater than or about 0.025% can yield from about 50% to about 100% of microspores at a uninucleate stage of development (see lines indicated by arrows).

The importance of colchicine is also taught at Col 5, lines 61-64;

> *In a preferred embodiment, 3 ml of 0.3 M mannitol combined with 50 mg/l of ascorbic acid, silver nitrate and colchicine is used for incubation of anthers at 10° C. for between 10 and 14 days.*

at Col 9, lines 32-41;

> *The rate of embryoid induction was much higher with the synergistic preculture treatment consisting of a combination of stress factors, including a carbon source which may be capable of inducing starvation, a cold temperature and colchicine, than has previously been reported. An illustrative embodiment of the synergistic combination of treatments leading to the dramatically improved response rate compared to prior methods, is a temperature of about 10° C., mannitol as a carbon source, and 0.05% colchicine.*

and at Col 10, line 67 to Col 11, line 6.

> *The inventors have added colchicine in increasing concentrations during mannitol pretreatment prior to anther culture and microspore culture and achieved improved percentages.*
>
> *An illustrative embodiment of the combination of a chromosome doubling agent and preculture medium is one which contains colchicine. In a specific embodiment, the colchicine level is preferably about 0.05%.*

Furthermore, Genovesi does not teach or even suggest any alternative, other than using colchicine, for maintaining from about 50% to about 100% of microspores at a uninucleate stage of development. Specifically, Genovesi provides no teaching or suggestion that:

- altering preculture conditions may provide from about 50% to about 100% of uninucleate microspores without the use of colchicine; or more specifically
- lowering preculture temperatures to less than 10°C would alter or eliminate the disclosed requirement for colchicine.

Thus Genovesi provides no indication as to which of the preculture conditions may be altered (for example, time of preculture or ascorbic acid concentration or mannitol

concentration or temperature level) to eliminate the colchicine requirement, and yet still "maintain from about 50% to about 100% of microspores at a uninucleate stage of development" as presently claimed. Given the Genovesi disclosure, a person skilled in the art would have no expectation as to what could be done to eliminate the colchicine requirement, and further would reasonably expect that the colchicine requirement could not be eliminated. For example, Genovesi strongly suggest that their combination of factors are an improvement over the prior art as stated in Col 9, lines 53-58:

> *These results are better than those previously reported from isolated microspore culture using any of the separate pretreatments disclosed in this invention, rather than the combination of pretreatment factors which is an aspect of this invention.*

Accordingly, in view of the state of the art at the time of the present invention applicants' claimed temperature range of "from about 3°C to about 6°C" is surprising, inventive and provides an unexpected and significant benefit of being able "to maintain from about 50% to about 100% of microspores at a uninucleate stage of development" without the use of colchicine (a compound that is known to lead to developmental abnormalities and is toxic to microspores as acknowledged by Genovesi at Col 10, lines 64-67).

Therefore, independent claims 1, 18, 31, 32, and 33, and associated dependant claims, are not obvious having regard to Genovesi, alone or in combination with Kreuger.

Second Argument

Applicants maintain their position, as stated in previous correspondence dated January 21, 2003, that a person skilled in the relevant art would not be motivated to combine the teachings of Genovesi and Kreuger.

Genovesi and Kreuger differ in several important respects.

One example of a difference between Kreuger and Genovesi is that the methods of Kreuger pertain to somatic cells (somatic embryogenesis), whereas the methods of Genovesi pertain to anther cultures or microspore cultures (androgenesis). Microspore culture is well known to have different requirements than somatic cell culture. Therefore, applicants submit that upon reading Genovesi a person skilled in the art would search art pertaining to microspore culture or androgenesis, and would not be expected to search art pertaining to somatic cell culture or somatic embryogenesis. Accordingly, Genovesi and Kreuger are not analogous art and should not be combined.

Another example of a difference between Kreuger and Genovesi is that the organisms used in the teachings of Kreuger, *Daucus, Lycopersicon, Cucumis, Brassica*, and *Acacia* are all dicots, whereas the teachings of Genovesi clearly relate to monocots (for example, see Col 3, line 68, and Col 4, lines 2 and 17). It is well known in the art that methods established using dicots are not readily applicable to monocots. For example, *Agrobacterium*-mediated plant transformation methods that were developed using dicots, were not readily transferable to monocots. Similarly, response of monocots in tissue culture is known to differ relative to dicots (e.g. see paragraph spanning page 294-295, in Vasil K., and V Vasil, 1994, *'In Vitro* culture of Cereals and Grasses, in *Plant Cell and Tissue Culture*, TA Thorpe and IK Vasil, Eds Kluwer Acad. Publ., pp293-312; copy enclosed). Accordingly, even if Genovesi and Kreuger are considered analogous art, a person skilled in the art would not be motivated to apply the tissue culture conditions of Kreuger developed by experimentation solely on dicots, to the monocot plants disclosed by Genovesi.

Another example of a difference between Kreuger and Genovesi is that the methods of Kreuger occur at 22°C, while the methods of Genovesi require a pretreatment at 10°C. Kreuger does not exemplify any methods of microspore culture, and does not teach how to apply the disclosed embryogenesis methods, directed to somatic (diploid) tissues, to microspore (haploid) culture. More significantly, in view of the temperature difference between Kreuger and Genovesi, Kreuger does not teach or suggest that the disclosed somatic embryogenesis methods can be applied to any cells, somatic or otherwise, that have been pretreated at 10°C.

As explained in applicants previous response dated January 21, 2003, stress factors, such as cold temperature, are known to activate particular genes in a plant cell, and can thereby alter a cell's response to culture conditions in comparison to a cell that was not exposed to the stress factor. Kreuger does not describe the use of any stress factors, and provides no indication that the disclosed tissue culture methods could be applied to cells, somatic or otherwise, that have been pretreated with such stress factors. Accordingly, even if Genovesi and Kreuger are considered analogous art, a person skilled in the art would not be motivated to combine Genovesi and Kreuger as there is no expectation that the methods of Kreuger could induce embryogenesis in a cell type, somatic, microspore, or otherwise, that has been exposed to stress factors, such as pretreatment at 10°C, let alone the temperatures defined in the claims of the present application, 3°C to 6°C.

In summary, applicants submit that Genovesi and Kreuger are not analogous art. If considered analogous, then it is submitted that there is no motivation to combine the two references as they apply to different culture techniques (microspore v. somatic), different plant types (monocot v. dicot), teach different conditions (10°C v. room temp.), and do not suggest to one of skill in the art that the non-disclosed tissue and plant types, and culture conditions may be applied.

Even if Genovesi and Kreuger are combined, the presently claimed subject matter of independent claims 1, 18, 31, 32, and 33, a argued above, is not taught or suggested in Genovesi, Kreuger, alone or in combination, and are therefore not obvious in view of this art. Claims 2-14, and 19-21 are free of Genovesi and Kreuger, alone or in combination, by incorporating inventive features from the independent claims by virtue of dependency.

Accordingly, withdrawal of Examiner's rejection of claims 1-14, 18-21 and 31-33 under 35 U.S.C. 103(a), alleging obviousness having regard to the combination of Genovesi and Kreuger, is respectfully requested.

In Item 7, the Examiner has rejected dependent claims 25-27 under 35 U.S.C. 103(a) as being unpatentable over the combination of Genovesi, Kreuger, and Chang et al.

The Chang et al disclosure generally relates to methods of genetic transformation. Applicants traverse Examiner's rejection for, at least, the reason that Genovesi and Kreuger, alone or in combination, do not render the independent claims of the present invention obvious (as explained above), and Chang et al fail to make up for the above-stated deficiencies of Genovesi and/or Kreuger. As claims 25-27 depend ultimately from claim 1, they include the limitations defined in this claim. Accordingly, withdrawal of Examiner's rejection of dependent claims 25-27 under 35 U.S.C. 103(a) is respectfully requested.

In Item 8, the Examiner has rejected dependent claims 13-17 under 35 U.S.C. 103(a) as being unpatentable over the combination of Genovesi, Kreuger, and Hu et al. As acknowledged by Examiner, Hu et al do not teach a pretreatment temperature of from about 3°C to about 6°C. Furthermore, Hu et al do not teach or suggest a method "to maintain from about 50% to about 100% of microspores at a uninucleate stage of development" without using colchicine. Therefore, applicants traverse Examiner's rejection for, at least, the reason that Genovesi and Kreuger, alone or in combination, do not render the independent claims of the present invention obvious (as explained above), and Hu et al fail to make up for the above-stated deficiencies of Genovesi and/or Kreuger. As claims 13-17 depend ultimately from claim 1 they include the limitations of this claim. Accordingly, withdrawal of

Examiner's rejection of dependent claims 13-17 under 35 U.S.C. 103(a) is respectfully requested.

It is respectfully submitted that the above-identified application is now in a condition for allowance and favorable reconsideration and prompt allowance of these claims are respectfully requested. Should the Examiner believe that anything further is desirable in order to place the application in better condition for allowance, the Examiner is invited to contact applicants' undersigned attorney at the telephone number listed below.

Respectfully submitted,

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